Subcutaneous phosphatidylcholine (PC) has been used effectively in the nonsurgical treatment of localized fat deposits in the abdomen, neck, arms, thighs, and lower eyelid fat pads, and has become an increasingly popular technique. It has been administered through superficial injections with the goal of destroying adipocytes. The lipases released from the adipocytes by means of PC produce a local breakdown of fat that is then discharged through the liver and metabolized via β-oxidation. It is known that PC causes chemical melting of the fat cells, but there has been no experimental study or clinical data collected about the effects of PC on peripheral nerves.

The aim of this study was to investigate the local effect of PC on the peripheral nervous tissue of rats.

METHODS

Twenty adult Lewis rats weighing between 200 and 300 g were divided into 2 experimental groups (n = 10). In group 1, animals received an intrafascicular injection of 0.1 mL PC (Lipostabil 250 mg/5 mL) with a 30-gauge needle into the left posterior tibial nerve. In group 2, as a negative control group, 0.1 mL normal saline was injected intrafascicularly respectively. After the operation, rats were evaluated on days 7, 14, and 21 with walking track analysis. On day 21, all the animals were sacrificed and the left tibial nerves were taken for histologic study. Light and electron microscopic studies, along with morphometric analysis, were performed.

RESULTS: According to the tibial nerve indices, there were no signs of nerve damage observed in either of the groups, and there was no statistical difference between the groups (P > .05). The nerves that received PC and saline injections could not be distinguished grossly and appeared similar to segments of the nerve that did not come in contact with either solution. The number and diameter of fibers, the thickness of the myelin, and the percentage of neural tissue were comparable with normal controls. According to these analyses, there were no statistical differences between the 2 groups (P > .05).

CONCLUSIONS: This study demonstrates that in a rat model, even direct intraneural injection of PC causes no damage. This information should encourage people to consider broader applications of PC.

BACKGROUND: Subcutaneous phosphatidylcholine (PC) injection has become a popular technique for treating localized fat accumulation. Some clinical studies reported minor local soft tissue complications, such as ecchymosis, edema, and pain. However, there are no data on how PC affects the peripheral nervous tissue.

OBJECTIVE: To investigate the local effect of PC on the peripheral nervous tissue of rats.

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CONCLUSIONS: This study demonstrates that in a rat model, even direct intraneural injection of PC causes no damage. This information should encourage people to consider broader applications of PC.
After the surgical procedures, the rats were evaluated on days 7, 14, and 21 with walking track analysis. At the end of day 21, all animals were sacrificed for histologic study.

**Surgical Procedure**

General anesthesia was induced with intramuscular ketamine hydrochloride (40 mg/kg) and chlorpromazine (40 mg/kg) on all animals. The left sciatic nerve was exposed through a gluteal muscle-splitting incision using sterile technique. The point of injection was below the sciatic nerve branch to the semimembranous and adductor magnus muscles. Using the operating microscope (OPMI; Carl Zeiss AG, Oberkochen, Germany), 1-mL syringes were used and 0.1 mL of PC or physiologic saline was injected carefully through a 30-gauge needle intramuscularly into the nerve fascicle itself on the sciatic nerve near the first muscular branch. The wounds were closed with 4-0 silk sutures after completing the procedures. The animals were taken to separate cages containing 2 rats each and maintained on standard rat chow and water ad libitum.

**Walking Track Analysis**

On days 7, 14, and 21, all the animals were given conditioning trials on an 8.2- to 42-cm walking track darkened at one end. Analysis was also carried out in all the rats just before surgical procedures, for comparison with normative data. After being taken, footprints were randomly measured according to the formula of Bain et al. Tibial nerve indices were assessed at the end of the study to evaluate possible nerve morbidity.

**Histologic Studies**

Twenty-one days after the walking track analysis, the animals were sacrificed by deep ether anesthesia and craniocervical dislocation technique. Left tibial nerves were harvested and processed for histologic and morphometric examination. The entire tibial nerve was removed en bloc, the injection site was identified, and the nerves were excised starting 1 cm proximally and continuing 1 cm distally to injection site. The neural tissue was fixed in 2.5% (w/v) glutaraldehyde, postfixed with osmium tetroxide, and embedded in Araldite 502 (Polysciences Inc, Warrington, PA). The samples were immersed in 10% neutral buffered formalin overnight. Tissues were embedded in paraffin and cross-sectioned at 5 μm. Sections were stained with toluidine blue. The number of axons was counted under light microscopy. Sections were examined on a transmission electron microscope (Jeol 1200 EX II; Jeol Ltd, Tokyo, Japan). The number and diameter of fibers, the thickness of the myelin, and the percentage of neural tissue were assessed at high magnification (×10,000).

**Statistical Analysis**

Results were analyzed statistically with the Statistical Package for Social Sciences (version 11.0; SPSS, Inc, Chicago, IL). When analyzing variables, descriptive statistics (mean and standard deviation) were used. In addition, Mann–Whitney U and Kruskal–Wallis tests were used to analyze the differences between variables. Results were evaluated in 95% confidence limits and P < .05 was considered statistically significant.

**RESULTS**

**Walking Track Analysis**

None of the rats died during the study period. Foot ulceration or automutilation were not observed. Preoperative tibial nerve indices were similar to normative data. Mean and standard deviations of tibial nerve indices were calculated for all groups and all intervals individually (Table 1). There was a statistically significant difference between day 7 and other time periods for both groups. There was not a statistically significant difference between the 14- or 21-day time periods for either group. The results of day 7 measurements were not significantly different between the PC and control-sham groups (P > .05). According to tibial nerve indices, there was no sign of nerve damage in any of the groups, and there was no statistical difference between the groups (P > .05).

**Light and Electron Microscopic Findings**

When the nerves were harvested, the nerves that received PC and saline injections could not be distinguished grossly and appeared similar to segments of the nerve that did not come in contact with either solution. This grossly normal appearance was noted. Sections of the nerve taken proximally and distally from the site of injection and at the site of injection for both PC injection and saline injection groups retained a normal architecture, could not be distinguished, and showed no evidence of direct neurotoxicity (Figure 1). Endoneural damage was not noticed. The endoneurial vessels and perineurium appeared intact. Axon numbers that had been calculated under light microscopy were analyzed. According to these analyses there were no statistical differences between the groups (P > .05; Table 2). Under electron microscopy, there was no demyelination, cellular infiltrate, inflammatory, or vascular reaction seen at any point along the nerve. In addition, the number and diameter of fibers, the thickness of the myelin, and the percentage of neural tissue injected with PC and saline were comparable to each other (Figure 2).

**Table 1. Tibial function indexes of groups in the walking track analysis results**

<table>
<thead>
<tr>
<th>Group 1 (PC)</th>
<th>Group 2 (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative</td>
<td>−1.6 ± 3.7</td>
</tr>
<tr>
<td>Day 7</td>
<td>−16.8 ± 8.4</td>
</tr>
<tr>
<td>Day 14</td>
<td>−2.1 ± 3.5</td>
</tr>
<tr>
<td>Day 21</td>
<td>−1.9 ± 4.5</td>
</tr>
</tbody>
</table>
DISCUSSION

PC, a lecithin extracted from soybeans, was initially used in emergencies for the treatment of atheromatous plaques in cardiac diseases and in the prophylaxis and therapy for fat embolisms and liver diseases.\textsuperscript{11,12} Recently, it has also become one of the most commonly used drugs for mesotherapy and lipolysis.\textsuperscript{13}

PC is the active drug in the commercial preparation of Lipostabil. It is composed primarily of PC and sodium deoxycholate, a bile salt used to solubilize the natural phospholipid in water. Rotunda et al\textsuperscript{14} reported that PC causes cell membrane lysis and fat disruption in cell cultures. Proposed mechanisms of action of PC include emulsification and transport of triglycerides from fat cells, detergent action that acts to open cell membranes, or stimulation of lipase activity.\textsuperscript{15} Furthermore, through its biophysical properties, locally injected PC could cause local liquefaction mimicking hyperthermia, thus mobilizing fat deposits.\textsuperscript{2,16}

Previous reports indicated that PC could be suitable as purported “fat-dissolving” injections. Palmer et al\textsuperscript{17} assessed retrospectively treatment outcomes and adverse effects associated with subcutaneous phosphatidylcholine use. They reported that localized adverse effects, such as swelling, erythema, burning, pain, tenderness, and bruising, were described by most patients as very mild or mild. The total incidence of systemic side effects was 3%: diarrhea, nausea, dizziness/light-headedness, and intermenstrual bleeding were described by most patients as very mild or mild. Only minimal unexpected, unusually severe, or prolonged adverse reactions, commonly including pain and/or swelling, were reported.\textsuperscript{17} Furthermore, some minor complications were reported in the literature by different authors, including transient erythema, minimal ecchymosis, and transient inflammatory reactions.\textsuperscript{7,18} Despite its attraction, the safety and efficacy of PC remain ambiguous to some patients and physicians.\textsuperscript{19,20} Because it is injected locally into the mesoderm or subcutaneous tissue, the possibility exists of its inadvertent injection into a peripheral nerve.

Selection of the appropriate parameter to measure neural degeneration is very important, and functional assessment is mandatory for experimental peripheral nerve studies. The rat sciatic nerve has been frequently used in experimental studies because it is indistinguishable from human peripheral nerves histologically.\textsuperscript{21–23} Typically, an agent that is neurotoxic causes damage over a considerable distance both proximal and distal to the site of injection.\textsuperscript{24,25}

Among the evaluation methods, walking analysis, light and electron microscopic studies, and morphometric analysis are reliable methods for evaluating changes of the neural tissue. The accuracy of these methods has been reported previously.\textsuperscript{26,27} To assess nerve function, conduction studies can be performed. However, the walking track assessment technique may be considered an overall functional assessment because walking requires complex motor unit activity coordinated by cortically integrated sensory feedback. Therefore, we preferred the walking track assessment technique to nerve conduction studies.\textsuperscript{27,28}

For both groups, there was a sta-

**Table 2. Myelinated axon counts of tibial nerves**

<table>
<thead>
<tr>
<th>No. of myelinated axons</th>
<th>Group 1 (PC)</th>
<th>Group 2 (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1356</td>
<td>1457</td>
</tr>
<tr>
<td>2</td>
<td>1457</td>
<td>1258</td>
</tr>
<tr>
<td>3</td>
<td>1246</td>
<td>1279</td>
</tr>
<tr>
<td>4</td>
<td>1276</td>
<td>1292</td>
</tr>
<tr>
<td>5</td>
<td>1244</td>
<td>1283</td>
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<tr>
<td>6</td>
<td>1385</td>
<td>1302</td>
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<tr>
<td>7</td>
<td>1245</td>
<td>1265</td>
</tr>
<tr>
<td>8</td>
<td>1282</td>
<td>1290</td>
</tr>
<tr>
<td>9</td>
<td>1267</td>
<td>1276</td>
</tr>
<tr>
<td>10</td>
<td>1243</td>
<td>1264</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1300.1 ± 74.0</td>
<td>1296.6 ± 58.0</td>
</tr>
</tbody>
</table>

**Figure 1.** Light microscopy views of nerve in (A) group 1 (PC) and (B) group 2 (control) show nerve fibers and retention of normal architecture and could not be distinguished. There was no evidence of direct neurotoxicity (toluidine blue stain; original magnification, ×100).
is of potential further clinical importance.

therapeutic doses of PC does not result in neurotoxicity and the percentage of neural tissue were comparable with normal controls. It did not disturb the myelin or diameter of the nerve fibers, the thickness of the myelin, and the percentage of neural tissue were comparable with normal controls. It did not disturb the myelin or any other ultrastructural features besides axon counts.

As a result, the finding that intrafascicular injection of therapeutic doses of PC does not result in neurotoxicity is of potential further clinical importance.

CONCLUSIONS

Our results provide a basis for preclinical investigations of PC in a sciatic neural model. We showed in the present study that therapeutic doses of PC did not have a harmful effect on neural tissue. These results further support the current evidence that PC is a safe modality for the treatment of a wide range of disorders.

DISCLOSURES

The authors have no disclosures with respect to the contents of this article.

REFERENCES

14. Rotunda AM, Suzuki H, Moy RL, Kolodney MS. Detergent effects of sodium deoxycholate are a major feature of an injectable phosphatidyl-


